CHAPTER 3A

3A.1 Introduction

Difficulties in the interpretation of intermittent significance found in the production data highlighted the limitations of the use of repeated measures analysis. To further investigate the effects of calcium presentation method on production parameters alternative analytical measures were sought. A method and the appropriate software (AS Restricted maximum likelihood (ASReml)) for analysing growth curves typified by the production data obtained here, became available mid-1998 at the time of the second draft of this thesis. ASReml (Gilmour et al., 1998) estimates variance components under a general mixed model by restricted maximum likelihood (Reml). Some of the production data obtained in the previous chapter was modelled by spline regression. Spline regression should be the applied procedure for the following reasons; 1. splines are preferable because the response is highly non-linear and not readily represented by a parametric function; 2. splines are non-parametric and the curves are derived predominantly from the data and reflect local and global changes over the duration of the experiment. For these data, a parametric model would be determined by all the data and its predictive ability at a particular time can be influenced by the data remote to this time; 3. preference should be given for data-driven rather than model-driven responses.

3A.2 Statistical design and analysis

To remove intermittent significant results at non-physiologically important points, profiles over time of body weight and mean calcium intake (Experiments 3.1 and 3.2), egg production and weights, gross energy crude protein and phosphorus intakes (Experiment 3.2) were modelled by spline regression taking into account the correlations due to repeated measures from each bird. These data were analysed by the ASReml programme (Gilmour et al., 1998). The initial model for each variate included all two way interactions of strain, feed and calcium method and week number and non-significant terms were deleted from the model in a stepwise regression procedure. The principles of calculating the spline curves are given in Welham et al. (1998).

3A.3 Results

3A3.1 Experiment 1

The re-analysis of the daily calcium intake (Figure 3A.1) produced significant (P < 0.05) interactions between calcium and week (time), feed and week (time), calcium and strain
and calcium and feed. The profiles over time differed for each feed and calcium method and the separation of profiles for each calcium method differed between strains. The separation of calcium profiles depended on the feed type, especially for the strain B (CB) birds. The strain A (HB) birds displayed a greater difference in their response to the different calcium methods across the feeds than did strain B.

![Graph showing calcium intake over age for strains A and B](image)

Figure 3A.1. Daily calcium intake (g/kg body weight) profiles during rearing of two strains of birds (A and B) fed a compound (Co) or choice (Ch) form of a standard diet and provided with calcium as either ground limestone (Ca 1), limestone grit available ad libitum (Ca 2) or limestone grit available every second day (Ca 3). Vertical error bar indicates the pointwise confidence interval around the mean curves.

### 3A3.2 Experiment 2

There was a significant \(P < 0.05\) calcium method x spline (week) interaction but the spline curves, revealed no practical difference in total eggs produced per bird. Egg weights were greater in strain A than strain B but the responses to time were similar for both strains.

Daily calcium intake (Figure 3A.2) showed a significant \(P < 0.05\) calcium x spline interaction. The birds fed the Ca 2 diet had a significantly \(P < 0.05\) higher calcium intake profile and maintained a greater increase in calcium intake through the peak production period than those fed either the Ca 1 or Ca 3 diets. The mean separation between the profiles revealed that the calcium intake of birds on the Ca 1 and Ca 3 diets was similar and the birds
on the Ca 2 diet ate more calcium than those on the Ca 1 diet. The mean profile separations are as follows;

\[
\begin{align*}
\text{Ca 1 - Ca 3} & = 0.084 \pm 0.26 \text{ g/kg body weight, } t = 0.32 \\
\text{Ca 2 - Ca 1} & = 0.841 \pm 0.26 \text{ g/kg body weight, } t = 3.14
\end{align*}
\]

![Graph showing calcium intake over age](image)

Figure 3A.2. Daily calcium intake (g/kg body weight) profiles during lay of hens provided with calcium as either ground limestone (Ca 1), limestone grit available ad libitum (Ca 2) or limestone grit available every second day (Ca 3). Vertical error bar indicates the pointwise confidence interval around the mean curves.

The greater gross energy intake in the strain B birds (Figure 3A.3) was explained as a significant mean difference as follows;

\[
\text{Strain B - Strain A} = 0.049 \pm 0.0026 \text{ MJ/kg body weight, } t = 13.75
\]

Crude protein intake involved feed method x week and strain x week interactions whereby the compound fed birds had a greater (P < 0.05) mean intake than the choice fed birds and strain B birds a greater (P < 0.05) mean intake than strain A birds. The response on each feed type and for each strain was different over time. Phosphorus intake followed that
for crude protein intake and the calcium method component was not significant (P > 0.05) in influencing intake.

Figure 3A.3. Daily gross energy intake (MJ/kg body weight) profiles during lay of two strains of birds (A and B). Vertical error bar indicates the pointwise confidence intervals surrounding the mean curves.

3A.4 Discussion

The spline regression, allowed for a simpler interpretation of the results, removing the erratic significant differences at non-physiologically important points. Furthermore, using the mean separation of significant results allowed a quick determination of intake differences over the course of each experiment. The reduction in calcium intake in the choice fed birds offered grit every second day may place the bird in danger of suffering a poor reserve of skeletal calcium in the critical period at point of lay. Clearly, the different profiles indicate that the local bird suffers this reduction in calcium intake in rear to a greater degree than the imported bird. However, the greater energy intake in the local bird may allow it to offset this low calcium intake especially when the greater energy intake is considered in the compounded feed. In the laying phase, the different profiles of calcium intakes are fully emphasised and indicate that the birds offered the particulate calcium source daily have a greater intake of calcium through the important period of point-of-lay to peak production. These birds then maintain a greater calcium intake throughout the production period. This may allow for an optimisation of calcium use in these birds as indicated by the greater eggshell weight found in these birds in Chapter 3.